SHORT COMMUNICATION

Plant Signaling & Behavior 6:12, 1897-1903; December 2011; ©2011 Landes Bioscience

Meta-analysis of the expression profiles of the Arabidopsis ESCRT machinery

Lynn G.L. Richardson and Robert T. Mullen*

Department of Molecular and Cellular Biology; University of Guelph; Guelph, ON Canada

Keywords: Arabidopsis, (co-)expression, endosome, E-northern, ESCRT, microarray, multivesicular body

The Endosomal Sorting Complex Required for Transport (ESCRT) machinery is a set of multi-protein complexes that are well conserved among all eukaryotes and mediate a remarkable array of cellular processes including late endosome/multivesicular body (MVB) formation, retroviral particle release and membrane abscission during cytokinesis. While the molecular mechanisms underlying ESCRT function have been relatively well characterized in yeasts and mammals, far less is known about ESCRT in plants. In this study, we utilized publicly-available microarray, massively parallel signature sequencing (MPSS) and proteome data sets in order to survey the expression profiles of many of the components of the *Arabidopsis thaliana* ESCRT machinery. Overall, the results indicate that ESCRT expression in Arabidopsis is highly dynamic across a wide range of organs, tissues and treatments, consistent with the complex interplay that likely exists between the spatial and temporal regulation of the ESCRT machinery and the diverse array of roles that ESCRT participates in during plant growth and development.

ESCRT consists of ~20 soluble proteins that are probably best known for their role in the recognition, concentration and packaging of mono-ubiquitinated, membrane-bound proteins within the intralumenal vesicles of endosomal MVBs, leading to the eventual degradation of these membrane proteins upon fusion of the MVB with the lysosome/vacuole. To accomplish this task, ESCRT proteins are sequentially recruited from the cytosol to the surface of the late endosomal membrane via a series of specific protein-protein interactions that results in the assembly of at least four heteromeric protein subcomplexes (ESCRT-0, -I, -II, -III). ESCRT subcomplex disassembly is subsequently regulated, in part, by an AAA-type ATPase referred to as Vps4 (vacuole protein-sorting 4) and its various associated regulatory proteins.[1-3]

Overall, the breadth of information that exists regarding ESCRT has been garnered primarily from studies with yeast and mammalian cultured cells; however, most of the components of ESCRT are present throughout all eukaryotic taxa, with the exception of ESCRT-0 apparently being absent in plants and other non-opisthokonts (e.g., trypanosomes, Dictyostelium, Chlamydomonas etc.).[4-6] Interestingly, components of ESCRT-III are present also in Archaea,[7] supporting the functional importance of ESCRT, or at least portions thereof, among evolutionarily diverse organisms.[8] It is also now well established that, in addition to the role of ESCRT in MVB biogenesis and membrane protein degradation via the endocytic pathway, ESCRT participates in a number of other key cellular events, including autophagy,[9] membrane abscission during cytokinesis,[10] retroviral budding,[11] and *tombusvirus* replication.[12]

In light of these new and unexpected functions for ESCRT there is a growing interest in understanding the overall, more global organization and regulation of ESCRT in plants. Toward this end, recent analyses of the ESCRT protein-protein interaction network in Arabidopsis—based mainly on the yeast two-hybrid assay—revealed that the majority of these interactions are those that also take place between their counterparts in yeasts and/or mammals,[13-15] suggesting that the molecular mechanisms underlying ESCRT function are evolutionarily conserved. On the other hand, the Arabidopsis ESCRT interactome also possesses a number of unique interactions that, when considering the fact that many of the ESCRT components in plants exist as multiple isoforms,[4,5,16] may reflect the functional plasticity and distinct regulation of the plant ESCRT system overall.

Here we extend our and other's recent, more global studies of the Arabidopsis ESCRT machinery[13-15] by taking advantage of the vast array of publicly-available Arabidopsis gene and protein (peptide) expression data sets and web-based bioinformatics resources to analyze the expression profiles of many of the Arabidopsis ESCRT components across a variety of different organs, tissues and treatments.

As shown in **Figure 1A**, electronic (E-)northern analyses revealed that almost all of the Arabidopsis ESCRT genes represented on the ATH1 whole-genome chip[17] are expressed in a wide variety of organs and/or tissues, as well as at various stages of growth and development, consistent with ESCRT serving a number of essential and general roles (e.g., MVB biogenesis, receptor downregulation, cell division, etc.,) throughout the plant life-cycle.[18,19] Notably, the expression levels of most of the

*Correspondence to: Robert Mullen; Email: rtmullen@uoguelph.ca
Submitted: 08/04/11; Revised: 09/07/11; Accepted: 09/07/11
DOI: 10.4161/psb.6.12.18023

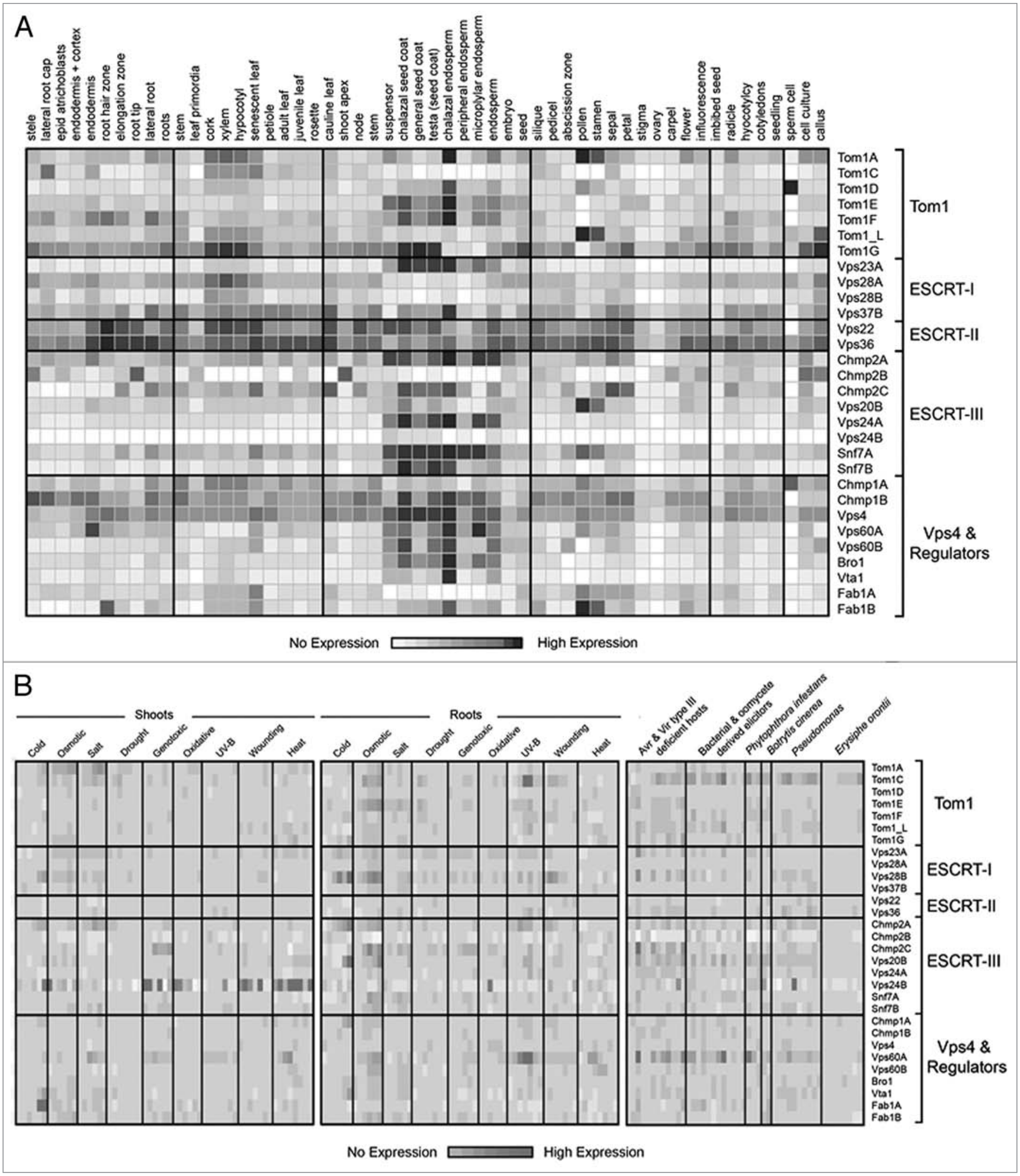

Figure 1. For figure legend, see page 1899.

ESCRT genes were usually highest in seed-specific tissues (e.g., seed coat and endosperm) (**Fig. 1A**), consistent with the known importance of ESCRT for embryo and seedling development.[20,21]

Several ESCRT genes also displayed prominent male reproductive-tissue-specific expression profiles. For example, the highest levels of *Tom1A*, *Tom_L*, *Vps20B* and *Fab1B* expression were in

Figure 1 (See opposite page). Microarray-based expression profiles of Arabidopsis ESCRT genes in various tissues and treatments. Publicly-available Arabidopsis microarray (E-northern) expression data sets were explored for selected Arabidopsis ESCRT genes (refer to Table 1 presented in ref. 14 for the list of known and putative Arabidopsis ESCRT genes examined in this study) in either (A) various organ and/or tissue types using the GENEVESTIGATOR Meta-Analyzer-Plant Organs database[26] (www.genevestigator.com/gv/) or (B) under different conditions and treatments in shoots or roots and pathogen infections using the AtGenExpress series database[38,39] (www.arabidopsis.org/portals/expression/microarray/ATGenExpress.jsp), and with the tools hosted at the BioArray Resource (BAR) (www.bar.utoronto.ca).[40] E-northern expression patterns were expressed as log-transformed values normalized to the controls and formatted into heat maps using the Meta Analyzer tool at BAR. Note that the E-northern data for some of the known ESCRT genes was not available, since these genes are not present on the ATH1 whole genome chip.[16] Keys at the bottom right of (A and B) display the correlation between shading and scaled log-fold changes in expression.

		Tom1							ESCRT-I				ESCRT-II	
		Tom1A	Tom1C	Tom1D	Tom1E	Tom1F	Tom_L	Tom1G	Vps23A	Vps28A	Vps28B	Vps37B	Vps22	Vps36
Tom1	Tom1A	-	0.14	0.46	0.04	0.23	0.47	0.43	0.44	0.22	0.35	0.08	0.12	0.01
	Tom1C	0.14	-	0.12	0.04	0.36	0.28	0.07	0.39	0.08	0.30	0.34	0.07	0.37
	Tom1D	0.46	0.12	-	0.00	0.12	0.49	0.32	0.33	0.18	0.31	0.20	0.17	0.01
	Tom1E	0.04	0.04	0.00	-	0.16	0.03	0.00	0.04	0.00	0.04	0.00	0.03	0.08
	Tom1F	0.23	0.36	0.12	0.16	-	0.24	0.05	0.23	0.09	0.18	0.28	0.09	0.26
	Tom_L	0.47	0.28	0.49	0.03	0.24	-	0.48	0.43	0.43	0.42	0.41	0.30	0.10
	Tom1G	0.43	0.07	0.32	0.00	0.05	0.48	-	0.30	0.29	0.29	0.09	0.10	0.00
ESCRT-I	Vps23A	0.44	0.39	0.33	0.04	0.23	0.43	0.30	-	0.13	0.62	0.20	0.12	0.09
	Vps28A	0.22	0.08	0.18	0.00	0.09	0.43	0.29	0.13	-	0.25	0.20	0.15	0.00
	Vps28B	0.35	0.30	0.31	0.04	0.18	0.42	0.29	0.62	0.25	-	0.20	0.26	0.06
	Vps37B	0.08	0.34	0.20	0.00	0.28	0.41	0.09	0.20	0.20	0.20	-	0.21	0.34
ESCRT-II	Vps22	0.12	0.07	0.17	0.03	0.09	0.30	0.10	0.12	0.15	0.26	0.21	-	0.12
	Vps36	0.01	0.37	0.01	0.08	0.26	0.10	0.00	0.09	0.00	0.06	0.34	0.12	-
ESCRT-III	Chmp2A	0.23	0.28	0.22	0.10	0.41	0.29	0.09	0.45	0.06	0.51	0.26	0.32	0.14
	Chmp2B	0.09	0.30	0.23	0.00	0.23	0.21	0.02	0.18	0.09	0.14	0.44	0.20	0.25
	Chmp2C	0.14	0.14	0.21	0.11	0.37	0.20	0.01	0.22	0.03	0.28	0.28	0.32	0.23
	Vps20B	0.27	0.23	0.19	0.14	0.29	0.42	0.13	0.36	0.16	0.41	0.35	0.29	0.14
	Vps24A	0.07	0.06	0.05	0.31	0.19	0.09	0.00	0.13	0.00	0.17	0.05	0.18	0.16
	Vps24B	0.01	0.00	0.00	0.10	0.05	0.01	0.00	0.00	0.10	0.03	0.00	0.04	0.01
	Snf7A	0.11	0.06	0.21	0.03	0.20	0.22	0.04	0.10	0.11	0.17	0.32	0.33	0.08
	Snf7B	0.19	0.25	0.30	0.11	0.27	0.36	0.09	0.34	0.08	0.40	0.34	0.41	0.21
Vps4 & Regulators	Chmp1A	0.33	0.25	0.42	0.02	0.30	0.61	0.27	0.44	0.34	0.51	0.51	0.33	0.08
	Chmp1B	0.16	0.13	0.17	0.01	0.38	0.26	0.04	0.12	0.14	0.14	0.38	0.28	0.09
	Vps4	0.23	0.12	0.27	0.07	0.28	0.27	0.09	0.20	0.02	0.17	0.23	0.10	0.18
	Vps60A	0.32	0.28	0.18	0.10	0.42	0.41	0.09	0.50	0.17	0.42	0.31	0.27	0.18
	Vps60B	0.31	0.08	0.32	0.07	0.16	0.53	0.27	0.30	0.31	0.43	0.29	0.52	0.06
	Bro1	0.60	0.30	0.49	0.02	0.45	0.51	0.38	0.43	0.16	0.29	0.26	0.13	0.10
	Vta1	0.48	0.33	0.19	0.03	0.32	0.23	0.29	0.42	0.10	0.33	0.07	0.01	0.03
	Fab1A	0.15	0.07	0.16	0.02	0.09	0.09	0.09	0.07	0.04	0.07	0.10	0.15	0.10
	Fab1B	0.33	0.08	0.24	0.02	0.27	0.23	0.11	0.15	0.08	0.16	0.15	0.37	0.11

		ESCRT-III								Vps4 & Regulators								
		Chmp2A	Chmp2B	Chmp2C	Vps20B	Vps24A	Vps24B	Snf7A	Snf7B	Chmp1A	Chmp1B	Vps4	Vps60A	Vps60B	Bro1	Vta1	Fab1A	Fab1B
Tom1	Tom1A	0.23	0.09	0.14	0.27	0.07	0.01	0.11	0.19	0.33	0.16	0.23	0.32	0.31	0.60	0.48	0.15	0.33
	Tom1C	0.28	0.30	0.14	0.23	0.06	0.00	0.06	0.25	0.25	0.13	0.12	0.28	0.08	0.30	0.33	0.07	0.08
	Tom1D	0.22	0.23	0.21	0.19	0.05	0.00	0.21	0.30	0.42	0.17	0.27	0.18	0.32	0.49	0.19	0.16	0.24
	Tom1E	0.10	0.00	0.11	0.14	0.31	0.10	0.03	0.11	0.02	0.01	0.07	0.10	0.07	0.02	0.03	0.02	0.02
	Tom1F	0.41	0.23	0.37	0.29	0.19	0.05	0.20	0.27	0.30	0.38	0.28	0.42	0.16	0.45	0.32	0.09	0.27
	Tom_L	0.29	0.21	0.20	0.42	0.09	0.01	0.22	0.36	0.61	0.26	0.27	0.41	0.53	0.51	0.23	0.09	0.23
	Tom1G	0.09	0.02	0.01	0.13	0.00	0.00	0.04	0.09	0.27	0.04	0.09	0.09	0.27	0.38	0.29	0.09	0.11
ESCRT-I	Vps23A	0.45	0.18	0.22	0.36	0.13	0.00	0.10	0.34	0.44	0.12	0.20	0.50	0.30	0.43	0.42	0.07	0.15
	Vps28A	0.06	0.09	0.03	0.16	0.00	0.10	0.11	0.08	0.34	0.14	0.02	0.17	0.31	0.16	0.10	0.04	0.08
	Vps28B	0.51	0.14	0.28	0.41	0.17	0.03	0.17	0.40	0.51	0.14	0.17	0.42	0.43	0.29	0.33	0.07	0.16
	Vps37B	0.26	0.44	0.28	0.35	0.05	0.00	0.32	0.34	0.51	0.38	0.23	0.31	0.29	0.26	0.07	0.10	0.15
ESCRT-II	Vps22	0.32	0.20	0.32	0.29	0.18	0.04	0.33	0.41	0.33	0.28	0.10	0.27	0.52	0.13	0.01	0.15	0.37
	Vps36	0.14	0.25	0.23	0.14	0.16	0.01	0.08	0.21	0.08	0.09	0.18	0.18	0.06	0.10	0.03	0.10	0.11
ESCRT-III	Chmp2A	-	0.27	0.66	0.57	0.39	0.01	0.46	0.70	0.57	0.30	0.34	0.64	0.48	0.30	0.26	0.03	0.28
	Chmp2B	0.27	-	0.36	0.19	0.03	0.00	0.40	0.45	0.38	0.33	0.12	0.19	0.22	0.17	0.05	0.27	0.32
	Chmp2C	0.66	0.36	-	0.43	0.47	0.03	0.54	0.66	0.45	0.36	0.42	0.48	0.43	0.20	0.07	0.05	0.28
	Vps20B	0.57	0.19	0.43	-	0.31	0.02	0.37	0.51	0.60	0.27	0.23	0.63	0.49	0.33	0.24	0.01	0.24
	Vps24A	0.39	0.03	0.47	0.31	-	0.04	0.21	0.36	0.14	0.10	0.24	0.38	0.26	0.07	0.03	0.00	0.09
	Vps24B	0.01	0.00	0.03	0.02	0.04	-	0.01	0.00	0.01	0.01	0.00	0.03	0.04	0.00	0.01	0.00	0.04
	Snf7A	0.46	0.40	0.54	0.37	0.21	0.01	-	0.55	0.49	0.35	0.23	0.33	0.47	0.14	0.02	0.06	0.32
	Snf7B	0.70	0.45	0.66	0.51	0.36	0.00	0.55	-	0.54	0.29	0.34	0.49	0.58	0.25	0.11	0.10	0.24
Vps4 & Regulators	Chmp1A	0.57	0.38	0.45	0.60	0.14	0.01	0.49	0.54	-	0.42	0.34	0.55	0.57	0.37	0.20	0.05	0.30
	Chmp1B	0.30	0.33	0.36	0.27	0.10	0.01	0.35	0.29	0.42	-	0.19	0.33	0.24	0.24	0.05	0.15	0.38
	Vps4	0.34	0.12	0.42	0.23	0.24	0.00	0.23	0.34	0.34	0.19	-	0.27	0.23	0.33	0.12	0.03	0.15
	Vps60A	0.64	0.19	0.48	0.63	0.38	0.03	0.33	0.49	0.55	0.33	0.27	-	0.48	0.34	0.25	0.02	0.24
	Vps60B	0.48	0.22	0.43	0.49	0.26	0.04	0.47	0.58	0.57	0.24	0.23	0.48	-	0.29	0.11	0.07	0.28
	Bro1	0.30	0.17	0.20	0.33	0.07	0.00	0.14	0.25	0.37	0.24	0.33	0.34	0.29	-	0.59	0.16	0.35
	Vta1	0.26	0.05	0.07	0.24	0.03	0.01	0.02	0.11	0.20	0.05	0.12	0.25	0.11	0.59	-	0.04	0.16
	Fab1A	0.03	0.27	0.05	0.01	0.00	0.00	0.06	0.10	0.05	0.15	0.03	0.02	0.07	0.16	0.04	-	0.33
	Fab1B	0.28	0.32	0.28	0.24	0.09	0.04	0.32	0.24	0.30	0.38	0.15	0.24	0.28	0.35	0.16	0.33	-

Figure 2. Co-expression analysis of Arabidopsis ESCRT genes. Multi-gene co-expression analysis of selected Arabidopsis ESCRT genes was performed using CressExpress[41] (www.cressexpress.org) and data obtained from the publicly-available Arabidopsis microarray (E-northern) expression data sets. Pearson's correlation coefficients (r values) between all ESCRT gene pairs are indicated, with those values reflecting positive co-expression ($r \geq 0.37$) at the whole plant level shaded in gray and those that also displayed an increased number of significant expression correlations ($r \geq 0.37$) in embryo and seed-specific tissues highlighted in bold. Similar results for Arabidopsis ESCRT co-expression at the whole plant level were obtained using other multi-gene co-expression analysis programs, including the PRIMe: Correlated Gene Search[42] (data not shown). Likewise, the co-expression values for the putative rice (*Oryza sativa)* ESCRT machinery,[4] based on the Rice Olig Array Database coexpression tool,[43] were also mostly variable overall at the whole plant level (data not shown), suggesting that the highly dynamic expression of the ESCRT machinery is a shared feature between dicots and monocots.

the pollen and stamens, while the highest levels of *Tom1D* expression were in sperm cells (**Fig. 1A**). These results are in line with other Arabidopsis comparative transcriptome studies,[22,23] wherein these tissues displayed enriched expression of genes involved in receptor protein signaling, vesicle trafficking and membrane transport, and all of which likely involve (directly or indirectly) ESCRT.

It was also notable that when comparing the E-northern expression profiles of Arabidopsis ESCRT genes according to the complexes in which their respective protein products are thought to function, only the ESCRT-II-related genes, *Vps22* and *Vps36*, appeared to be largely ubiquitously expressed and at relatively high levels (**Fig. 1A**). By contrast, the expression profiles and levels of most other ESCRT-related genes varied considerably. Indeed, other than perhaps for ESCRT-III along with Vps4 [also referred to as AtSKD1 (*Arabidopsis thaliana* suppressor of K^+ transport growth defect 1)] [24] and its regulators, co-expression values for the Arabidopsis ESCRT machinery overall were quite variable, with few significant correlations in transcript expression levels within or between most of the different ESCRT subcomplexes at the whole plant level (**Fig. 2**). Moreover, almost all of the Arabidopsis ESCRT components that exist as multiple isoforms, including *Vps23A* (also referred to as *ELC*),[25] *Vps23B*, *Chmp2A-C and Vps24A/B*, etc., displayed highly divergent expression patterns (**Figs. 1A and 2**), suggesting that, despite their high sequence identities[4,5,16] and their similar protein-protein interaction networks,[13-15] functional redundancy of these proteins is unlikely. Instead, these observations reinforce the premise that compared with other evolutionarily diverse organisms, the considerable expansion of ESCRT gene families in plants likely reflects organ/tissue-specific functions or differences in protein-binding specificity.

In support of this notion, when the co-expression values for the Arabidopsis ESCRT machinery were analyzed in embryo and seed-specific tissues [where the expression of many of the ESCRT genes are at their highest (**Fig. 1A**)], several components, including many of those that exist as multiple isoforms, displayed an increased number of significant expression correlations (**Fig. 2**). However, absent from these embryo and seed-specific co-expression results was co-expression for certain ESCRT components that have been reported previously to physically interact using protein-protein interaction assays,[13-15] or ectopically expressed in plant cells.[15]

Probably the most obvious group of ESCRT-associated components in Arabidopsis to undergo marked gene expansion is the Tom1 gene family, which has nine members (*Tom1A-G*). These proteins have been proposed to perform an MVB cargo recognition role in place of ESCRT-0. That is, similar to other non-opisthokonts, plants have no apparent homologs for the mammalian and yeast ESCRT-0 components Hrs/Vps27 and STAM/HseI;[4,5] the two key factors that function in mammals and yeasts in the initial recognition of ubiquitinated cargo (membrane) proteins and the subsequent recruitment of ESCRT-I to the endosomal surface.[1-3] This lack of Vps27/Hrs and HseI/STAM in plants is thought to reflect the fact that the nature of ESCRT cargo protein selection and ESCRT-I recruitment in plants is mechanistically distinct, such that other ubiquitin-binding proteins, namely members of the Tom1 protein family, serve as an alternate interface for these two roles.[4,5] Based on this presumption and also the divergent (co-)expression profiles observed for several members of plant Tom1 family (**Figs. 1A and 2**), it is tempting to speculate further that at least some of these proteins have evolved to function in a tissue-specific manner and/or interact with certain components of ESCRT-I[13] or select ESCRT cargo proteins.[20,21] Interestingly, many of the non-ESCRT genes that are co-expressed with ESCRT genes in embryo and seed-specific tissues encode membrane proteins involved in a wide array of cellular processes, and include drug, ion, amino acid, oligopeptide and sugar transporters, as well as predicted transmembrane receptors (**Table 1**). Of course, while it remains to be determined whether any of these proteins are bona fide ESCRT (cargo) substrates, these findings are consistent with the general role of ESCRT in the regulated degradation of membrane proteins and perhaps highlight yet-to-be studied roles for ESCRT in other physiological processes in plants.

Also shown in **Figure 1B**, additional surveys of the Arabidopsis E-northern expression databases for various abiotic stress treatments of shoots and roots or pathogen and elicitor treatments of leaves revealed that at least a few ESCRT genes displayed pronounced changes in their expression in response to these different treatments. Most notably, the putative ESCRT-III gene *Vps24B* was highly upregulated in shoots subjected to either genotoxic, oxidative, wounding or heat stress, as well as in late-stage (i.e., 48 h) Pseudomonas-infected leaves (**Fig. 1B**). Likewise, both *Tom1C* and *Vps60A* genes displayed significant increases in expression levels in roots subjected to UV-B stress and in response to almost all pathogen infections and elicitor treatments examined (**Fig. 1B**). Similar increases in *Tom1C* and *Vps60A* expression were also observed in various Arabidopsis (non-ESCRT) mutant gene backgrounds, including those with defects in pathogen resistance, i.e., surveys of the GENEVESTIGATOR genotype E-northern database[26] revealed that both *Tom1C* and *Vps60A*, as well as various other ESCRT genes, displayed significant increases in expression in, among others, mutants defective in local and systemic acquired resistance (*sid2*),[27] pathogen response signaling (*cpr5*),[28] or plant innate immune responses (*mpk4*) [29] (data not shown). However, whether these results further reflect novel roles for components of the plant ESCRT machinery, perhaps during unique or general plant stress responses, remains to be determined. Nonetheless, this is an intriguing possibility given the apparent role of ESCRT in *tombusvirus* RNA replication. That is, similar to ability of enveloped RNA viruses, such as HIV, to appropriate certain constituents of ESCRT to execute their budding from infected mammalian cells,[30] the *tombusvirus*, tomato bushy stunt virus replication proteins appear to exploit the ESCRT machinery,[12,31] in order to mediate the structural reorganization of peroxisomes into unique MVB-like compartments where at viral RNA replication takes place.[32]

We also analyzed the expression profiles for several known and putative ESCRT components across different organs using the MPSS and peptide proteome databases.[33,34] As shown in **Figure 3**, MPSS and peptide proteome analyses revealed many of the same general and organ/tissue-specific expression patterns for those ESCRT genes that were analyzed using the E-northern data sets (**Fig. 1**) and, for at least *Vps23A* and Vps4, that have been analyzed also using RT-PCR and protein blotting, respectively.[24,25] On the other hand, some conspicuous differences in the expression patterns of ESCRT genes (proteins) were also found. For instance, based on MPSS results, among the highest levels of expression for *Tom1_L*, *Chmp2A* and the putative *ESCRT-III* genes, *Snf7A* and *Snf7B*, were in the roots, whereas each of these genes, based on E-northerns, displayed relatively low levels of expression throughout various root tissues (**Figs. 1A and 3A**). Similarly, the relative protein expression levels for some ESCRT components based on the peptide proteome (**Fig. 3B**) were varied compared with that of their corresponding mRNA (microarray and/or MPSS) expression levels in the various plant organs/tissues examined (e.g., *Tom1F*, *Snf7A/B*, *Chmp1A* and *Bro1*). However, these apparent differences may be due, at least in part, to inherent variations between these different technology platforms; for example, gene-specific cDNA or oligonucleotide-sequence vs. peptide-sequence sampling, (sub)tissue/organ sampling, filtering stringency and tag-position bias.[35]

Taken together, the expression profiling data presented here suggest that the regulation of ESCRT in Arabidopsis is highly complex, including a dynamic array of organ/tissue-specific and stress-related expression patterns, as well as apparent transcriptional and post-transcriptional controls. However, this complex regulation of Arabidopsis ESCRT is perhaps not surprising given a number of factors, including: (1) the considerable expansion of many of the ESCRT gene families in plants compared with other eukaryotes,[4,5,15] (2) the sophisticated hierarchical- and stoichiometric-dependent manner in which the various multi-protein ESCRT subcomplexes, their associated regulatory proteins, as well as ubiquitin, appear to operate in concert,[1-3,36] (3) the unique and dynamic nature of the plant endosomal pathway overall,[17,18,37] and (4) the ever expanding number of roles that the ESCRT machinery, or at least portions thereof, participates in throughout plant growth and development.[8] Undoubtedly these and other important aspects of the plant ESCRT machinery will be the focus over the coming years of more detailed experimental studies and the results presented here should thus serve as a useful resource for elucidating the molecular mechanisms by which ESCRT functions in plants.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to R.T.M. L.G.L.R. is the recipient of an NSERC CGS-D Postgraduate Scholarship. We thank Geoffrey Lum for his assistance with the co-expression analysis of the rice ESCRT machinery.

Table 1. Non-ESCRT genes co-expressed with the ESCRT gene network in Arabidopsis embryo and seed tissues[a]

AGI[b]	Gene Description[c]
AT3G49630	Unknown function
AT1G23300	MATE efflux family protein; antiporter activity, drug transmembrane transporter activity
AT2G14610	PATHOGENESIS-RELATED GENE 1
AT1G23830	Unknown function
AT3G57100	Unknown function
AT2G06130	Transposable element gene
AT1G51210	UDP-Glycosyltransferase superfamily protein
AT1G63770	Peptidase M1 family protein; metallopeptidase activity, zinc ion binding
AT3G02260	ATTENUATED SHADE AVOIDANCE 1; auxin polar transport, ubiquitin-protein ligase activity
AT1G48510	Surfeit locus 1 cytochrome *c* oxidase biogenesis protein
AT2G02620	Cysteine/Histidine-rich C1 domain family protein; zinc ion binding; intracellular signaling pathway
AT4G38830	Cysteine-rich receptor-like protein kinase
AT4G30940	BTB/POZ domain with WD40/YVTN repeat-like protein; voltage-gated potassium channel activity
AT5G05300	Unknown function
AT3G46150	Unknown function
AT1G71680	Transmembrane amino acid transporter family protein
AT1G28220	Member of a family of proteins related to PUP1, a purine transporter
AT1G57670	Toll-Interleukin-Resistance domain family protein; transmembrane receptor activity
AT3G25280	Major facilitator superfamily protein; oligopeptide transporter activity
AT4G04690	Protein ubiquitination; ubiquitin-dependent protein catabolism; ubiquitin-protein ligase activity
AT2G14700	Unknown function
AT2G13620	Member of Putative Na^+/H^+ antiporter family
AT1G51750	Transposable element gene
AT2G23270	Unknown function
AT5G28580	Transposable element gene
AT4G17450	Transposable element gene
AT2G35080	ATP binding; aminoacyl-tRNA ligases
AT1G27160	Valyl-tRNA synthetase/valine-tRNA ligase-related
AT2G34040	Apoptosis inhibitory protein 5
AT1G13610	Alpha/β-Hydrolases
AT3G55100	ABC-2 type transporter family protein
AT1G57943	Purine base transmembrane transporter activity
AT4G26340	F-box/RNI-like/FBD-like domains-containing protein; C
AT1G65870	Disease resistance-responsive (dirigent-like protein) family protein
AT3G10100	Transposable element gene
AT2G24920	Transposable element gene
AT1G62280	SLAC1 HOMOLOG 1; cellular ion homeostasis, plasma membrane transporter activity
AT2G05390	Transposable element gene
AT2G01580	Unknown protein
AT1G28460	AGAMOUS-LIKE 59; sequence-specific DNA-binding transcription factor activity
AT2G16120	Polyol/monosaccharide transporter 1
AT1G57906	Unknown protein
AT5G52050	MATE efflux family protein; antiporter activity, drug transmembrane transporter activity
AT2G17330	CYTOCHROME P450 51A1; putative obtusifoliol 14-α demethylase

[a]Identification of non-ESCRT genes that were co-expressed with the Arabidopsis ESCRT gene network was performed using CressExpress[38] and data obtained from the publicly-available Arabidopsis microarray (E-northern) expression data sets specific for embryo and seed tissues. Genes shown are those that were most significantly co-regulated [Pearson's correlation coefficients reflecting positive co-expression ($r \geq 0.5$)] with at least 18 of the 19 ESCRT genes examined (i.e., ESCRT genes present on the ATH1 whole genome chip and listed in **Fig. 2**). [b]Arabidopsis gene identifier (AGI) number based on The Arabidopsis Information Resource (TAIR)[44] (www.arabidopsis.org). [c]Description of gene product function(s) based on TAIR.

Table 1. Non-ESCRT genes co-expressed with the ESCRT gene network in Arabidopsis embryo and seed tissues[a] (continued)

AGI[b]	Gene Description[c]
AT3G60760	Unknown function
AT3G50560	NAD(P)-binding Rossmann-fold superfamily protein
AT2G13630	F-box associated ubiquitination effector family protein
AT5G55330	MBOAT (membrane bound O-acyl transferase) family protein
AT3G60720	Plasmodesmal protein; may be involved in the intercellular movement of molecules through the plasmodesmata
AT4G10990	Transposable element gene
AT1G57600	MBOAT (membrane bound O-acyl transferase) family protein
AT1G73700	MATE efflux family protein; antiporter activity, drug transmembrane transporter activity
AT3G04960	Molecular chaperone, heat shock protein
AT1G29350	Kinase-related protein of unknown function
AT1G76430	Pht1;9, a member of the Pht1 family of phosphate transporters
AT5G01550	LECTIN RECEPTOR KINASE A4.1

[a]Identification of non-ESCRT genes that were co-expressed with the Arabidopsis ESCRT gene network was performed using CressExpress[38] and data obtained from the publicly-available Arabidopsis microarray (E-northern) expression data sets specific for embryo and seed tissues. Genes shown are those that were most significantly co-regulated [Pearson's correlation coefficients reflecting positive co-expression ($r \geq 0.5$)] with at least 18 of the 19 ESCRT genes examined (i.e., ESCRT genes present on the ATH1 whole genome chip and listed in **Fig. 2**). [b]Arabidopsis gene identifier (AGI) number based on The Arabidopsis Information Resource (TAIR)[44] (www.arabidopsis.org). [c]Description of gene product function(s) based on TAIR.

References

1. Roxrud I, Stenmark H, Malerød L. ESCRT & Co. Biol Cell 2010; 102:293-318; PMID:20222872; DOI:10.1042/BC20090161.
2. Hurley JH, Hanson PI. Membrane budding and scission by the ESCRT machinery: it's all in the neck. Nat Rev Mol Cell Biol 2010; 11:556-66; PMID:20588296; DOI:10.1038/nrm2937.
3. Henne WM, Buchkovich NJ, Emr SD. The ESCRT pathway. Dev Cell 2011; 21:77-91; PMID:21763610; DOI:10.1016/j.devcel.2011.05.015.
4. Winter V, Hauser MT. Exploring the ESCRTing machinery in eukaryotes. Trends Plant Sci 2006; 11:115-23; PMID:16488176; DOI:10.1016/j.tplants.2006.01.008.
5. Leung KF, Dacks JB, Field MC. Evolution of the multivesicular body ESCRT machinery: retention across the eukaryotic lineage. Traffic 2008; 9:1698-716; PMID:18637903; DOI:10.1111/j.1600-0854.2008.00797.x.
6. Herman EK, Walker G, van der Giezen M, Dacks JB. Multivesicular bodies in the enigmatic amoeboflagellate *Breviata anathema* and the evolution of ESCRT 0. J Cell Sci 2011; 124:613-21; PMID:21266469; DOI:10.1242/jcs.078436.
7. Samson RY, Bell SD. Ancient ESCRTs and the evolution of binary fission. Trends Microbiol 2009; 17:507-13; PMID:19783442; DOI:10.1016/j.tim.2009.08.003.
8. Michelet X, Djeddi A, Legouis R. Developmental and cellular functions of the ESCRT machinery in pluricellular organisms. Biol Cell 2010; 102:191-202; PMID:20059450; DOI:10.1042/BC20090145.
9. Fader CM, Colombo MI. Autophagy and multivesicular bodies: two closely related partners. Cell Death Differ 2009; 16:70-8; PMID:19008921; DOI:10.1038/cdd.2008.168.
10. Caballe A, Martin-Serrano J. ESCRT machinery and cytokinesis: the road to daughter cell separation. Traffic 2011; 12:1318-26; PMID:21722282; DOI:10.1111/j.1600-0854.2011.01244.x.
11. Carlton JG, Martin-Serrano J. The ESCRT machinery: new functions in viral and cellular biology. Biochem Soc Trans 2009; 37:195-9; PMID:19143630; DOI:10.1042/BST0370195.
12. Barajas D, Jiang Y, Nagy PD. A unique role for the host ESCRT proteins in replication of Tomato bushy stunt virus. PLoS Pathog 2009; 5:1000705; PMID:20041173; DOI:10.1371/journal.ppat.1000705.
13. Shahriari M, Keshavaiah C, Scheuring D, Sabovljevic A, Pimpl P, Häusler RE, et al. The AAA-type ATPase AtSKD1 contributes to vacuolar maintenance of *Arabidopsis thaliana*. Plant J 2010; 64:71-85; PMID:20663085.
14. Richardson LGL, Howard ASM, Khuu N, Gidda SK, McCartney A, Morphy BJ, et al. Protein-protein interaction network and subcellular localization of the *Arabidopsis thaliana* ESCRT machinery. Front Plant Sci 2011; In press; DOI:10.3389/fpls.2011.00020.
15. Shahriari M, Richter K, Keshavaiah C, Sabovljevic A, Huelskamp M, Schellmann S. The Arabidopsis ESCRT protein-protein interaction network. Plant Mol Biol 2011; 76:85-96; PMID:21442383; DOI:10.1007/s11103-011-9770-4.
16. Mullen RT, McCartney AW, Flynn CR, Smith GST. Peroxisome biogenesis and the formation of multivesicular peroxisomes during tombusvirus infection: a role for ESCRT? Can J Bot 2006; 84:551-64; DOI:10.1139/b06-005.
17. Redman JC, Haas BJ, Tanimoto G, Town CD. Development and evaluation of an Arabidopsis whole genome Affymetrix probe array. Plant J 2004; 38:545-61; PMID:15086809; DOI:10.1111/j.1365-313X.2004.02061.x.
18. Otegui MS, Spitzer C. Endosomal functions in plants. Traffic 2008; 9:1589-98; PMID:18627577; DOI:10.1111/j.1600-0854.2008.00787.x.
19. Schellmann S, Pimpl P. Coats of endosomal protein sorting: retromer and ESCRT. Curr Opin Plant Biol 2009; 12:670-6; PMID:19836992; DOI:10.1016/j.pbi.2009.09.005.
20. Tian Q, Olsen L, Sun B, Lid SE, Brown RC, Lemmon BE, et al. Subcellular localization and functional domain studies of DEFECTIVE KERNEL1 in Maize and Arabidopsis suggest a model for aleurone cell fate specification involving CRINKLY4 and SUPERNUMERARY ALEURONE LAYER1. Plant Cell 2007; 19:3127-45; PMID:17933905; DOI:10.1105/tpc.106.048868.
21. Spitzer C, Reyes FC, Buono R, Sliwinski MK, Haas TJ, Otegui M. The ESCRT-related CHMP1A and B proteins mediate multivesicular body sorting of auxin carriers in Arabidopsis and are required for plant development. Plant Cell 2009; 21:749-66; PMID:19304934; DOI:10.1105/tpc.108.064865.
23. Borges F, Gomes G, Gardner R, Moreno N, McCormick S, Feijó JA, et al. Comparative transcriptomics of Arabidopsis sperm cells. Plant Physiol 2008; 148:1168-81; PMID:18667720; DOI:10.1104/pp.108.125229.
24. Haas TJ, Sliwinski MK, Martínez DE, Preuss M, Ebine K, Ueda T, et al. The Arabidopsis AAA ATPase SKD1 is involved in multivesicular endosome function and interacts with its positive regulator LYST-INTERACTING PROTEIN 5. Plant Cell 2007; 19:1295-312; PMID:17468262; DOI:10.1105/tpc.106.049346.
25. Spitzer C, Schellmann S, Sabovljevic A, Shahriari M, Keshavaiah C, Bechtold N, et al. The Arabidopsis elch mutant reveals functions of an ESCRT component in cytokinesis. Development 2006; 133:4679-89; PMID:17090720; DOI:10.1242/dev.02654.
26. Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W. GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol 2004; 136:2621-32; PMID:15375207; DOI:10.1104/pp.104.046367.
27. Wildermuth MC, Dewdney J, Wu G, Ausubel FM. Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 2001; 414:562-5; PMID:11734859; DOI:10.1038/35107108.
28. Kirik V, Bouyer D, Schöbinger U, Bechtold N, Herzog M, Bonneville JM, et al. CPR5 is involved in cell proliferation and cell death control and encodes a novel transmembrane protein. Curr Biol 2001; 11:1891-5; PMID:11728314; DOI:10.1016/S0960-9822(01)00590-5.
29. Rodriguez MC, Petersen M, Mundy J. Mitogen-activated protein kinase signaling in plants. Annu Rev Plant Biol 2010; 61:621-49; PMID:20441529; DOI:10.1146/annurev-arplant-042809-112252.
30. Weiss ER, Göttlinger H. The role of cellular factors in promoting HIV budding. J Mol Biol 2011; 410:525-33; PMID:21762798; DOI:10.1016/j.jmb.2011.04.055.
31. Barajas D, Nagy PD. Ubiquitination of tombusvirus p33 replication protein plays a role in virus replication and binding to the host Vps23p ESCRT protein. Virology 2010; 397:358-68; PMID:20004458; DOI:10.1016/j.virol.2009.11.010.
32. Mullen RT, Gidda SK. The role of peroxisomes in viral replication. In: Emergent Functions of the Peroxisome. Terlecky SR, Titorenko VI, Eds. Research Signpost 2009.

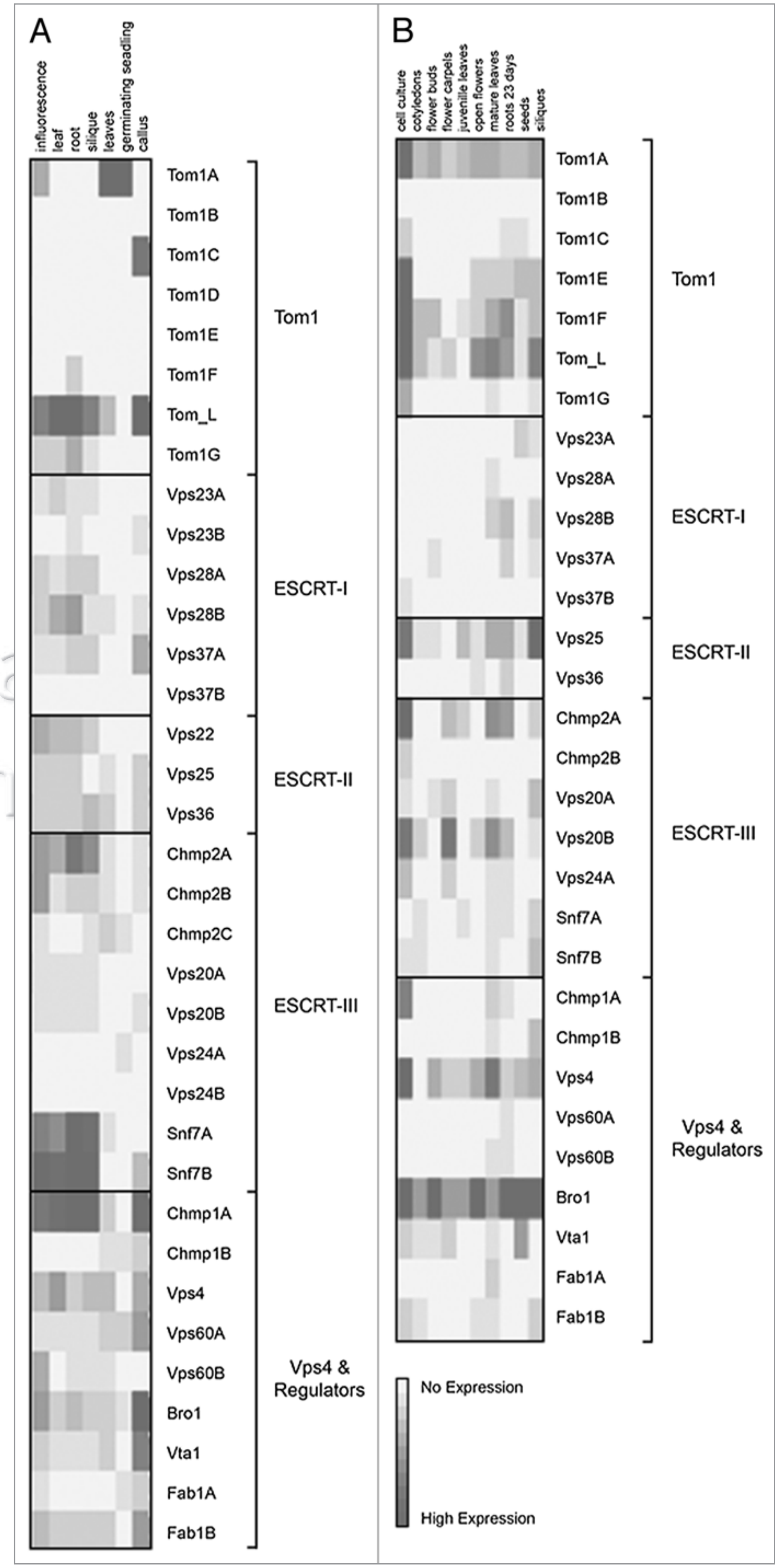

Figure 3. MPSS and peptide expression profiles of Arabidopsis ESCRT genes and proteins in various tissues. (A) MPSS analysis of selected Arabidopsis ESCRT genes in various tissue types was performed using the MPSS Plus website (mpss.udel.edu/at/).[33] (B) Peptide expression profiles for selected Arabidopsis ESCRT proteins in different tissues were based on the Arabidopsis peptide-to-proteome TAIR9 database (fgcz-pep2pro.uzh.ch/).[34] Both MPSS and peptide expression values (compared with controls) were normalized and formatted as heat maps using the DataMeta-Formatter tool at BAR.[40] The key at the bottom of (B) display the correlation between shading and scaled log-fold changes in expression.

33. Meyers BC, Tej SS, Vu TH, Haudenschild CD, Agrawal V, Edberg SB, et al. The use of MPSS for whole-genome transcriptional analysis in Arabidopsis. Genome Res 2004; 14:1641-53; PMID:15289482; DOI:10.1101/gr.2275604.
34. Baerenfaller K, Grossmann J, Grobei MA, Hull R, Hirsch-Hoffmann M, Yalovsky S, et al. Genome-Scale proteomics reveals *Arabidopsis thaliana* gene models and proteome dynamics. Science 2008; 320:938-41; PMID:18436743; DOI:10.1126/science.1157956.
35. Baginsky S, Hennig L, Zimmermann P, Gruissem W. Gene expression analysis, proteomics and network discovery. Plant Physiol 2010; 152:402-10; PMID:20018595; DOI:10.1104/pp.109.150433.
36. Shields SB, Piper RC. How ubiquitin functions with ESCRTs. Traffic 2011; 12:1306-17; PMID:21722280; DOI:10.1111/j.1600-0854.2011.01242.x.
37. Robinson DG, Jiang L, Schumacher K. The endosomal system of plants: charting new and familiar territories. Plant Physiol 2008; 147:1482-92; PMID:18678740; DOI:10.1104/pp.108.120105.
38. Kilian J, Whitehead D, Horak J, Wanke D, Weinl S, Batistic O, et al. The AtGen-Express global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. Plant J 2007; 50:347-63; PMID:17376166; DOI:10.1111/j.1365-313X.2007.03052.x.
39. Goda H, Sasaki E, Akiyama K, Maruyama-Nakashita A, Nakabayashi K, Li W, et al. The AtGenExpress hormone and chemical treatment data set: experimental design, data evaluation, model data analysis and data access. Plant J 2008; 55:526-42; PMID:18419781; DOI:10.1111/j.1365-313X.2008.03510.x.
40. Toufighi K, Brady SM, Austin R, Ly E, Provart NJ. The botany array resource: e-northerns, expression angling and promoter analyses. Plant J 2005; 43:153-63; PMID:15960624; DOI:10.1111/j.1365-313X.2005.02437.x.
41. Srinivasasainagendra V, Page GP, Mehta T, Coulibaly I, Loraine AE. CressExpress: A tool for large-scale mining of expression data from Arabidopsis. Plant Physiol 2008; 147:1004-16; PMID:18467456; DOI:10.1104/pp.107.115535.
42. Akiyama K, Chikayama E, Yuasa H, Shimada Y, Tohge T, Shinozaki K, et al. PRIMe: a Web site that assembles tools for metabolomics and transcriptomics. In Silico Biol 2008; 8:339-45; PMID:19032166.
43. Jung KH, Dardick C, Bartley LE, Cao P, Phetsom J, Canlas P, et al. Refinement of light-responsive transcript lists using rice oligonucleotide arrays: evaluation of gene-redundancy. PLoS ONE 2008; 3:3337; PMID:18836531; DOI:10.1371/journal.pone.0003337.
44. Poole RL. The TAIR database. Methods Mol Biol 2007; 406:179-212; PMID:18287693; DOI:10.1007/978-1-59745-535-0_8.